PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BP107363/IR	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/FI2003/000619	International filing date *(day/month/year)* 22.08.2003	Priority date *(day/month/year)* 23.08.2002
International Patent Classification (IPC) or national classification and IPC C07K 14/465		
Applicant Jyväskylän Yliopisto et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of _____ sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☒ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☒ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02.03.2004	25.11.2004
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INTERNATIONAL PRELI[redacted]ARY REPORT ON PATENTABILITY

Inter[redacted]al application No.
PCT/FI2003/000619

Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))
☐ publication of the international application (under Rule 12.4)
☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☒ the international application as originally filed/furnished
☐ the description:
pages _____ as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____
☐ the claims:
pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____
☐ the drawings:
pages _____ as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____
☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2003/000619

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

The priority is considered valid for the features disclosed in 'The Journal of Biological Chemistry, Volume 278, no. 4, 2003, Henri R. Nordlund et al: "Enhancing the Thermal Stability of Avidin", page 2476-page 2483"'. Therefore, this document is not included in the statement in Box V.

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Box No. IV Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:

   ☐ complied with.

   ☒ not complied with for the following reasons:

The application is considered to contain 3 inventions as follows:

1.1 Claims 1-2 (partly), 3-12, 13 (partly), 16, 19-21 (partly) are directed to a mutant of a biotin binding protein wherein the mutant has intermonomeric disulphide bridges in the tetramer. The mutants have increased thermostability compared to the wild type biotin binding protein.

1.2 Claims 15, 21 (partly) are directed to a thermally stable AVR4/5.

2. Claims 1-2 (partly), 13 (partly), 14, 18-21 (partly) are directed to a mutant of a biotin binding protein wherein a cysteine/intramonomeric disulpide bridges have been substituted/deleted.

3. Claim 17, 18 (partly), 21 (partly) are directed to a mutant of a biotin binding protein wherein asparagine 43 (glycosylation site) of AVR4/5 have been changed to glutamic acid.

The present application has been considered to contain 3 inventions which are not linked such that they form a single general inventive concept, as required by Rules 13.1, 13.2 and 13.3 PCT for the following reasons: .../...

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-2(part.),3-12,13(p.),15-16,19-21(p.)

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box IV

The prior art is represented by Reznik GO et al., Nature Biotechnology, Volume 14, August 1996, pages 1007-1011 (D1). D1 concerns streptavidin (a biotin binding protein) with intersubunit crosslinks. In one mutant, His at position 127 has been substituted by Cys, forming an intersubunit disulphide bond (Stv-C127). Stv-C127 shows enhanced thermal stability compared to a natural core streptavidin (see Figure 5). The formation of the disulphide bond had no effect on the biotinbinding ability (page 1008, left column, second paragraph). Thus, the concept of the present invention, i.e. to provide a mutant biotin binding protein having an improved thermal stability compared to the wild type, by forming intermonomeric disulphide bridges is already known in the prior art. Therefore, no unifying special technical feature has been found between the different inventions listed above, that is, between the different solutions of providing biotin binding proteins having improved properties compared to the wild type protein.

Invention 1.1 relates to the problem of providing a mutant biotin binding protein having an enhanced thermal stability compared to the wild type protein. This problem appears to be solved by introducing (substituting for) cysteine residues in order to form intermonomeric disulphide bridges.

Invention 1.2 relates to the problem of providing thermally stable AVR4/5 in general.

Invention 2 relates to the problem of providing a mutant biotin binding protein wherein naturally occurring cysteine residues have been substituted/deleted. The problem appears to be solved by changing cysteine 60 in AVR1,AVR3, AVR6 or AVR 7 or by deleting the four intramonomeric disulphide bridges in chicken avidin. It seems to be unclear which the improved properties compared to the wild type protein are.

Invention 3 relates to the problem of providing a mutant biotin binding protein having an altered glycosylation. The problem appears to be solved by substituting asparagine 43 in AVR4/5 for glutamic acid. It seems to be unclear which the improved properties compared to the wild type protein are, except for the known fact that deglycosylation may decrease non-specific binding. In the description (page 15, lines 22-24) it says that the glycosylation of Asn-43 had no marked influence on biotin binding, thermal stability or structural properties of AVR4/5.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box IV

In conclusion, therefore, the 3 group of claims are not linked by common or corresponding technical features and define different inventions not linked by a single general inventive concept. The application, hence does not meet the requirements of unity of invention as defined in Rule 13.1 and 13.2 PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2003/000619

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	5-13,16,19-20	YES
	Claims	1-4,15,21	NO
Inventive step (IS)	Claims		YES
	Claims	1-13,15-16,19-21	NO
Industrial applicability (IA)	Claims	1-13,15-16,19-21	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

This examination only concerns invention 1.

Invention 1:

Claims 1-2 (partly), 3-12, 13 (partly), 16 and 19-21 (partly) are directed to a mutant of a biotin binding protein wherein the mutant has intermonomeric disulphide bridges in the tetramer. The mutants have increased thermostability compared to the wild type biotin binding protein.

Claims 15 and 21 (partly) are directed to a thermally stable AVR4/5.

Documents cited in the International Search Report:
D1: Nature Biotechnology, Volume 14, 1996, Gabriel O. Reznik et al: "Streptavidins with intersubunit crosslinks have enhanced stability", page 1007-page 1011
D2: WO 9711183 A1
D3: FEBS Letters, Volume 467, 2000, Ari T. Marttila et al: "Recombinant NeutraLite Avidin: a non-glycosylated, acidic mutant of chicken avidin that exhibits high affinity for biotin and low non-specific binding properties"; page 31-page 36
D4: Biochem. J., Volume 363, 2002, Olli H. Laitinen et al: "Chicken avidin-related proteins show altered biotin-binding and physico-chemical properties as compared with avidin", page 609-page 617
D5: Nature, Volume 342, 16 November 1989, Masazumi Matsumura et al: "Substantial increase of protein stability by multiple disulphide bonds", page 291- page 293

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

D6: WO 0105977 A1
D7: The Journal of Biological Chemistry, Volume 263, No. 24, 1988, C. Nick Pace et al, "Conformational Stability and Activity of Ribonuclease T1 with Zero, One, and Two Intact Disulfide Bonds", pages 11820-11825
D8: BioEssays, Volume 8, No. 2, 1988, Thomas E. Creighton, "Disulphide Bonds and Protein Stability", pages 57-63
D9: Applied Biochemistry and Biotechnology, Volume 53, 1995, Edward A. Bayer et al, "Preparation of Deglycosylated Egg White Avidin", pages 1-9

Documents D1-D5 are considered to be of particular relevance for invention 1.

D1 and D2 disclose streptavidin mutants with intersubunit crosslinks, i.e. intermonomeric crosslinks. In one mutant, histidine at position 127 has been replaced with a cysteine, thus creating an intermonomeric disulphide bond. The formation of the disulfide bond had no effect on the biotin-binding ability. The mutant had an increased thermal stability compared to the native streptavidin. (D1: abstract; page 1008, left column, second paragraph; page 1009, left column, fourth column - page 1010, left column, first paragraph; D2: example 35, claim 37.)

Consequently, the subject matter claimed in claims 1-4 and 21 lacks novelty.

D3 discloses an avidin derivate, in which five out of eight arginine residues were replaced with neutral amino acids, two of the lysine residues were replaced by glutamic acid and the carbohydrate-bearing asparagine-17 residue was altered to an isoleucine. The resulting mutant is a non-glycosylated and acid form of avidin which exhibits superior properties compared to e.g. wild type avidin. (Abstract; table 1.)

Thus, D3 discloses an additional mutant biotin binding protein with improved properties. In view of D3, claims 1, 2 and 21 lacks novelty.

D4 has produced recombinant chicken avidin related proteins (AVRs) in order to analyse their properties. All AVRs, including AVR4/5, exhibited heat stability.

Thus, thermally stable AVR4/5 is known, and therefore the subject matter claimed in claim 15 lacks novelty. .../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

D1 is considered to be one document disclosing the closest prior art.

The mutant biotin binding proteins claimed in claims 5-13, 16 and 19-20 differ from the mutant disclosed in D1 in different ways, e.g. by specific mutations, specific avidin variants etc.

These differences have not been shown to give rise to any unexpected technical effect.

The problem to be solved is thus merely to provide alternative biotin binding mutants having increased thermostability compared to the wild type biotin binding protein.

It is not considered to require any inventive skill for a person skilled in the art, who is faced with the problem stated above, to obtain such novel biotin binding mutants with increased thermostability. The techniques that are required are techniques commonly used in his area of research. In addition, research on the structure of avidin and the AVRs has been done, see e.g. D4, which would guide the person skilled in the art to suitable positions for introducing disulfide bridges. Consequently, the subject matter claimed in claims 5-13, 16 and 19-20 is considered to lack an inventive step.

D2 is considered to be one additional document disclosing the closest prior art. This document can be used in the same manner as D1 in order to evaluate the inventive step of the subject matter claimed in claims 5-13, 16 and 19-20.

D5 discloses the facts that disulphide bonds can stabilise the native structure of proteins and gives the protein an increase in melting temperature. The increase in melting temperature from the individual disulphide bonds is approximately additive. D5 is considered to disclose the general state of the art.

Documents D6-D9 are considered to represent the general state of the art.

To summaries, the subject matter claimed in claims 1-4, 15 and 21 is lacks novelty. The subject matter claimed in claims 1-13, 15-16 and 19-21 is considered to lack an inventive step. The subject matter claimed in claims 1-13, 15-16 and 19-21 is consdiered to be industrially applicable.